

COVID-19 Pandemic Impacts on Humans Taking Antibiotics in China

Yu Hu, Xianping Wei, Qingqing Zhu, Lingxiangyu Li, Chunyang Liao,* and Guibin Jiang



Cite This: *Environ. Sci. Technol.* 2022, 56, 8338–8349



Read Online

ACCESS |



Metrics & More



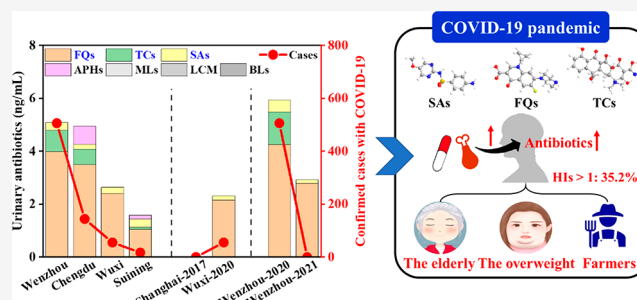
Article Recommendations



Supporting Information

ABSTRACT: The outbreak of the novel coronavirus 2019 (COVID-19) pandemic has resulted in the increased human consumption of medicines. Antibiotics are of great concern due to their adverse effects, such as increased bacterial resistance and dysbiosis of gut microbiota. Nevertheless, very little is known about the changes in self-medication with antibiotics during the COVID-19 pandemic and the resultant potential health risks. Herein, we examined the concentration profiles of some commonly used antibiotics in human urine collected from several geographical regions in China between 2020 and 2021. Antibiotics were found in 99.2% of the urine samples at concentrations ranging from not detected (nd) to 357 000 (median: 10.2) ng/mL. During the COVID-19 pandemic, concentrations of urinary antibiotics were remarkably higher than those found either before the pandemic or in the smooth period of the pandemic. Moreover, elevated levels of antibiotics were determined in urine samples from the regions with more confirmed cases. The exposure assessment showed that hazard index values >1 were determined in 35.2% of people. These findings show that human exposure to antibiotics increased during the COVID-19 pandemic, and further research is imperative to identify the public health risks.

KEYWORDS: COVID-19, antibiotics, urine, regional distribution, public health



1. INTRODUCTION

From the time of the outbreak of the novel coronavirus 2019 (COVID-19) in December 2019, over 247 million cases have been confirmed throughout the world, with more than 5.01 million deaths reported as of November 3, 2021.¹ Non-pharmaceutical interventions, such as social distancing and home quarantine, have been proven to be effective in preventing the spread of COVID-19.^{2–4} With concerns about cross-infection, humans prefer to purchase medicines either online or in the pharmacy for treating diseases, instead of going to see doctors in the hospital. It has also been reported that medicine sales in hospitals in China decreased by 13.4% during the time period from January to June 2020 compared to the same time duration in 2019.⁵ However, the online pharmacy sales of medicines between January and June 2020 were ~ 2 times higher than those at the same time period in 2019.⁵ When regarding similar symptoms, humans usually have to take a higher dose of medicines via self-medication at home than via intravenous administration in hospitals, due to the better bioavailability of medicines via intravenous injection ($\sim 100\%$).⁶ Among all medicines in the pharmacy, antibiotics have shown almost the highest average sales.^{7,8}

Antibiotics are commonly used as human medicines to prevent and treat infectious diseases.^{9,10} China is one of the countries that use antibiotics to the highest degree in the world.¹¹ A total of 162 000 tons of antibiotics were consumed in China in 2013, with 48% used for humans and 52% used for animals.¹¹ The widespread occurrence of antibiotics in various

environmental matrices in China has been documented, such as being found in surface water,^{12,13} sediment,^{12,13} sewage sludge,¹⁰ manure,¹⁰ poultry food,¹⁴ and aquatic products.^{15,16} Sources of human exposure to antibiotics include medications, dietary intake, and other sources. The health impacts of antibiotic exposure include toxic effects (such as nephrotoxicity and ototoxicity),¹⁷ increased bacterial resistance,¹⁸ and dysbiosis of gut microbiota.¹⁹ Bacterial resistance can be significantly amplified via the oral administration of antibiotics compared to other ingestion pathways.^{20,21} It is estimated that antibiotic resistance will contribute to 10 million deaths throughout the world in 2050.²² Dysbiosis of gut microbiota is associated with secondary metabolic diseases (such as obesity), allergy disorders, autoimmune diseases, and infectious diseases.¹⁹ With increasing concern about the health risk of antibiotic exposure, the Administrative Measures for Clinical Use of Antimicrobial Agents and the National Action Plan for Inhibiting Bacterial Resistance (2016–2020) were implemented in 2012 and 2016, respectively.^{23,24} The use of veterinary antibiotics has been observed to decrease by 57.0% from 2014 to 2018.²⁵ Furthermore, the percentage of

Received: November 10, 2021

Revised: March 31, 2022

Accepted: May 24, 2022

Published: June 8, 2022



Table 1. Urinary Concentrations (ng/mL) of 31 Commonly Used Antibiotics Grouped Into Seven Categories in General Populations from Four Cities in China ($n = 663$)^a

antibiotics	usage	n (%) ^b	P25 ^c	P50 ^c	P75 ^c	P95 ^c	maximum
sulfonamides ^d		573 (86.4)	0.0815	0.273	0.648	1.81	254
sulfamonomethoxine	PVA	26 (3.92)	— ^e	—	—	—	2.51
sulfamethoxazole	PVA	12 (1.81)	—	—	—	—	214
sulfaquinoxaline	VA	27 (4.07)	—	—	—	—	2.82
sulfameter	PVA	303 (45.7)	—	—	0.219	0.941	2.6
sulfamethazine	PVA	2 (0.302)	—	—	—	—	0.0356
sulfadiazine	PVA	26 (3.92)	—	—	—	—	2.17
sulfamethizole	PVA	1 (0.151)	—	—	—	—	0.0739
sulfachlorpyridazine	VA	6 (0.905)	—	—	—	—	0.669
trimethoprim	PHA	544 (82.1)	0.0519	0.174	0.341	0.862	38.9
fluoroquinolones ^d		529 (79.8)	0.344	2.57	6.86	29.4	20 900
ciprofloxacin	PVA	210 (31.7)	—	—	0.932	4.26	36.4
norfloxacin	PVA	380 (57.3)	—	0.95	2.81	10.4	171
ofloxacin	PVA	372 (56.1)	—	0.102	0.483	11.7	20 900
enrofloxacin	VA	138 (20.8)	—	—	—	0.42	47.3
difloxacin	VA	17 (2.56)	—	—	—	—	24.2
pefloxacin	PVA	149 (22.5)	—	—	—	0.737	23.3
fleroxacin	PHA	119 (17.9)	—	—	—	0.142	6.12
lomefloxacin	PVA	39 (5.88)	—	—	—	0.0605	15.9
macrolides ^d		233 (35.1)	—	—	0.0327	4.33	4880
roxithromycin	PHA	50 (7.54)	—	—	—	0.19	1530
clarithromycin	PVA	85 (12.8)	—	—	—	1.84	4880
erythromycin-H ₂ O	PVA	29 (4.37)	—	—	—	—	9.87
erythromycin	PVA	132 (19.9)	—	—	—	0.0562	29.6
tylosin	VA	19 (2.87)	—	—	—	—	0.386
tetracyclines ^d		386 (58.2)	—	0.244	1.19	8.88	580
oxytetracycline	PVA	317 (47.8)	—	—	0.245	1.28	98.7
chlortetracycline	PVA	114 (17.2)	—	—	—	0.927	18.4
doxycycline	PVA	265 (40)	—	—	0.24	2.3	289
tetracycline	PHA	274 (41.3)	—	—	0.294	2.41	290
lincosamides ^d		48 (7.24)	—	—	—	0.218	131
lincomycin	PVA	48 (7.24)	—	—	—	0.218	131
β -lactams ^d		236 (35.6)	—	—	15	138	357 000
penicillin-G	PVA	35 (5.28)	—	—	—	0.0532	2.24
amoxicillin	PVA	222 (32.9)	—	—	15	138	357 000
amphenicols ^d		259 (39.1)	—	—	0.781	4.55	732
florfenicol	VA	66 (9.95)	—	—	—	0.525	730
chloramphenicol	PVA	218 (32.9)	—	—	0.508	3.64	284
PVAs ^d		648 (97.7)	2.79	8.29	33.5	215	357 000
VAs ^d		222 (33.5)	—	—	0.105	1.3	730
PHAs ^d		616 (92.9)	0.164	0.389	0.867	6	1540
total ^d		658 (99.2)	3.27	10.1	38.2	263	357 000

^aVA, veterinary antibiotic; PVA, antibiotic preferred as veterinary antibiotic; PHA, antibiotic preferred as human antibiotic. ^b n , number of samples with positive detection (detection frequency, %). ^cP25, P50, P75, and P95 denote the 25th, 50th (median), 75th, and 95th percentile concentrations. ^dSum concentrations of antibiotics in the corresponding category. ^e—: Values below LOQ.

inpatients and outpatients who were prescribed antibiotics decreased from 55.2% and 17.2% in 2011 to 38.0% and 9.1% in 2017, respectively.²⁶ However, antibiotics are still dispensed in 83.6% of pharmacies in China without prescriptions, as shown by a nationwide, cross sectional study.^{8,27} The public awareness of the rational use of antibiotics is relatively weak in China. For example, an investigation in 2019 reported that only 9.7% of pharmacy customers had a good perception of antibiotics.²⁸ During the COVID-19 pandemic, more immoderate ingestion of antibiotics may have occurred because humans would purchase antibiotics in online pharmacies rather than go to see doctors in hospitals, as was previously mentioned. According to the behavioral insight research by

the World Health Organization (WHO), 79–96% of antibiotics have been inappropriately consumed in the European Region since the time of the COVID-19 pandemic.²⁹

The exposure of certain populations in Eastern China to antibiotics during 2012–2019 has been reported. For example, health risks based on microbiological effects were determined in 3.5% of elderly individuals,³⁰ 4.3% of pregnant women,³¹ 6% of children,³² and 7.2% of adults³³ in Eastern China due to antibiotic exposure. Despite this result, little is known about the impacts of the COVID-19 pandemic on the antibiotic exposure of humans in different regions of China. In this study, we measured the concentrations of 31 commonly used antibiotics in the first morning spot urine samples ($n = 663$)

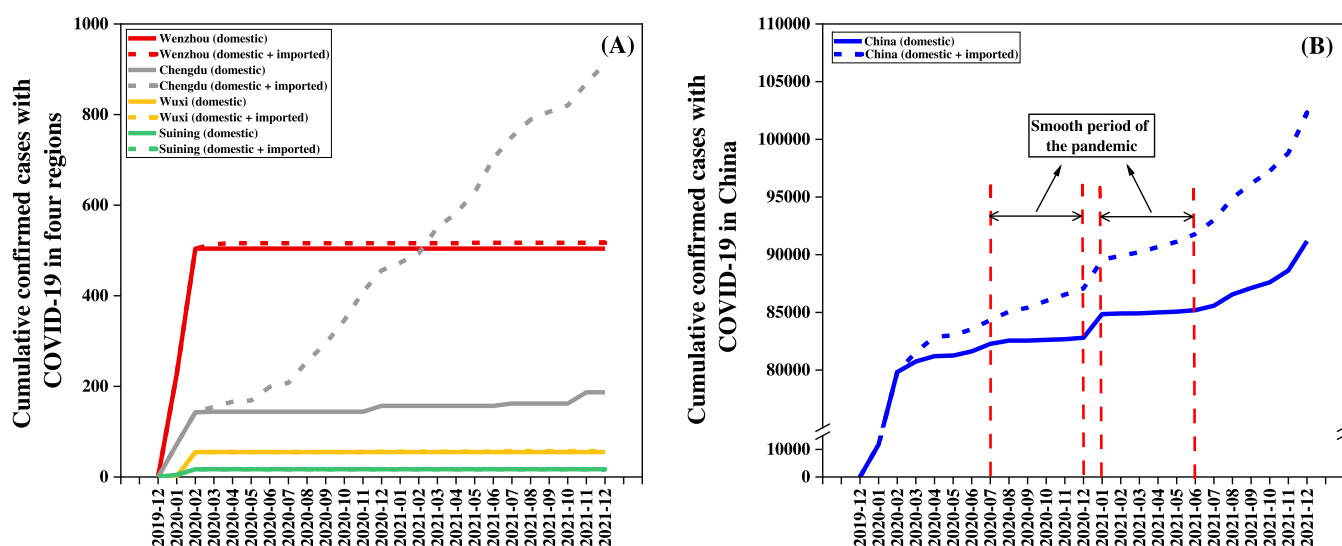


Figure 1. Cumulative confirmed cases with COVID-19 in (A) four regions in China and (B) in the whole of China from December 2019 to December 2021.

collected from general populations in several geographical regions of China and investigated the impacts of the COVID-19 pandemic on human exposure to antibiotics in these regions. Furthermore, other factors concerning exposure were discussed, including regional differences and various demographic parameters. This study fills a knowledge gap by identifying the variation in urinary antibiotics in humans and exploring the associations of urinary antibiotics with the COVID-19 pandemic.

2. MATERIALS AND METHODS

2.1. Chemicals. According to the consumption of antibiotics^{11,34} and previous studies reporting the occurrence of antibiotics in environmental and biological media in China,^{10,12,14–16,35} 31 commonly used antibiotics were analyzed in this study, which were grouped into seven categories, including nine sulfonamides, eight fluoroquinolones, five macrolides, four tetracyclines, two β -lactams, two amphenicols, and lincomycin (Table 1). Among these 31 antibiotics, 6 are veterinary antibiotics (VAs), 21 are preferred as VAs (PVAs), and 4 are preferred as human antibiotics (PHAs) (Table 1). Details of the chemicals and reagents are provided in the Supporting Information.

2.2. Urine Sample Collection. The first morning spot urine samples (~ 50 mL for each; $n = 663$) were collected from general populations in several cities of China from April to June 2020 and in June 2021, including Wenzhou city in Zhejiang Province, Wuxi city in Jiangsu Province, and Chengdu and Suining cities in Sichuan Province. The COVID-19 outbreak emerged in China at the beginning of 2020 and remained relatively smooth in the second half of 2020 and in the first half of 2021 (Figure 1B).³⁶ The smooth period of the pandemic comes with the end of the lockdown in China, which is represented by several events: (1) students completely returned to universities and colleges in China during August and September 2020³⁷ and (2) the cumulative confirmed cases remained nonincreased nationwide for more than 20 days for the first time, which was noted by the WHO on September 7, 2020.³⁸ In addition, a total of 639 million COVID-19 vaccines have been inoculated into Chinese people as of the end of May 2021.³⁹ In this way, the human safety of

outdoor activities (such as going to see doctors in hospitals) can be ensured, to some extent. Wenzhou was the second epicenter of the COVID-19 pandemic in China, and this designation was transferred from Wuhan (the first epicenter), with relatively more confirmed domestic cases than the other three cities until June 2020 (Figure 1A).^{40–42} Therefore, Wenzhou was selected as a representative city for sampling in 2020–2021 to examine the impacts of the COVID-19 pandemic on human exposure to antibiotics in China. The urine sampling was approved by the ethics committee of the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences.

All of the participants were voluntarily recruited from communities in the above-mentioned cities, which were randomly selected in Eastern and Western China. The stratified random sampling method was applied for recruitment in each region, with the advantages of having a good sample representation and small sampling errors.⁴³ The main districts/counties were selected in each city, and the number of humans recruited in each district was based on the proportion of the district's population to the city's total population. For the accuracy of the demographics for the health risk assessment, individuals with local residence time durations of more than 6 months were included, and those with psychiatric disorders or liver or kidney diseases were excluded. In this way, a broader community within the investigated regions can be reflected by the sample pool. All of the participants were asked to sign an informed consent form and complete a questionnaire in a face-to-face interview that included questions concerning demographics about age, gender, height, weight, occupation, and eating habits. Questionnaire surveys are commonly used to collect basic information in epidemic studies, although they have uncertainties due to the subjectivity of the self-reported information.^{34,44} For uncertain information about body height and weight, an on-site measurement was performed. Samples were categorized into different groups by the sampling year, region, age, occupation, gender, body mass index (BMI), and consumption frequency of eggs, milk, and meat^{32,45,46} (Tables S2 and S3). After sampling, all of the samples were transported to the laboratory on dry ice and stored at -20 °C until

analysis. The analysis of urine samples was completed within 4 months after sampling.

2.3. Sample Preparation. Samples were extracted according to previous methods, with some modifications.^{47,48} Briefly, 1.0 mL of the thawed urine was spiked with 50 ng of internal standards and then adjusted to an approximate pH of 7.0 by using 1.0 M ammonium acetate buffer (pH 9.0). After the addition of 15 μ L of β -glucuronidase aqueous solution ($\geq 85\,000$ units/mL) from *Helix pomatia* (type H-2; Sigma-Aldrich, St. Louis, MO), the mixture was vortexed and incubated at 37 $^{\circ}$ C overnight for hydrolysis. The hydrolyzed sample was centrifuged at 1857g for 10 min. The supernatant was then transferred and purified by an Oasis HLB cartridge (60 mg/3 mL; Waters, Milford, MA). The HLB cartridge was preconditioned with 1.5 mL of methanol and 1.5 mL of ultrapure water. After loading ~ 1.5 mL of the supernatant, the cartridge was washed with 1.5 mL of ultrapure water and 1.5 mL of 30% methanol in water to remove impurities and dried for ~ 20 min under vacuum. Analytes were eluted with 1.5 mL of methanol and 1.5 mL of acetonitrile. After concentration to 1 mL under a gentle stream of nitrogen and filtration via a 0.22 μ m nylon filter membrane (Shimadzu, Kyoto, Japan), 200 μ L of the eluate was transferred and concentrated to dryness. The residue was redissolved in 50 μ L of 10% methanol in water for ultraperformance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) analysis.

2.4. Instrumental Analysis. An Exion LC AD Series UPLC interfaced with an AB Sciex 5500 triple quadrupole mass spectrometer (Applied Biosystems, Framingham, MA) was used to determine the target 31 antibiotics. A BEH C18 column (100 \times 2.1 mm², 1.7 μ m; Waters) connected in series to a BEH C18 guard column (5 \times 2.1 mm², 1.7 μ m; Waters) was employed for the chromatographic separation. The column temperature was set at 40 $^{\circ}$ C. Ultrapure water containing 0.1% formic acid (A) and methanol containing 0.1% formic acid (B) were used as the mobile phase. The flow rate was 0.4 mL/min, and the sample injection volume was 5.0 μ L. The negative ionization multiple-reaction monitoring (MRM) mode was utilized for three target analytes (amoxicillin, florfenicol, and chloramphenicol), and the positive ionization MRM mode was utilized for other target analytes. In the negative ionization mode, the gradient of the mobile phase was programmed as follows: 0–1.0 min, 10% B–15% B; 1.0–3.0 min, 15% B–60% B; 3.0–4.0 min, 60% B; 4.0–4.5 min, 60% B–95% B; 4.5–6.0 min, 95% B; 6.0–6.1 min, 95% B–10% B; and 6.1–7.0 min, 10% B. The curtain gas and collision gas (both nitrogen) were 40 psi and 9 psi, respectively. The ion spray voltage was –4500 V, and the ion source temperature was 550 $^{\circ}$ C. In the positive ionization mode, the gradient was as follows: 0–2.5 min, 10% B–15% B; 2.5–5.5 min, 15% B–60% B; 5.5–6.0 min, 60% B–95% B; 6.0–8.0 min, 95% B; 8.0–8.1 min, 95% B–10% B; and 8.1–9.0 min, 10% B. The curtain gas and collision gas (both nitrogen) were 35 and 7 psi, respectively. The ion spray voltage was 5500 V, and the ion source temperature was 500 $^{\circ}$ C. The MS/MS parameters of the target analytes are presented in Table S1.

2.5. Quality Assurance and Quality Control (QA/QC). Two procedural blanks, two matrix-spiked samples, and duplicate samples were processed for each batch of 90 urine samples. Calibration standards were prepared in ultrapure water containing 10% methanol for each batch, with 10 concentrations ranging from 0.01 to 200 ng/mL. For every 20

samples, 10% methanol and the midpoint calibration standard were injected to monitor the carryover of antibiotics between samples and the drift of instrumental sensitivity.

Trace levels of trimethoprim (0.183 ng/mL) and erythromycin (0.0699 ng/mL) were detected in the procedural blanks. The background was subtracted in the quantification of trimethoprim and erythromycin. The average recoveries of spiked antibiotics in the urine were in the range of 48.1% (for florfenicol) to 155% (for ofloxacin; Table S4), and the coefficient variations of the antibiotics between duplicate samples were <15%. Good linearity was observed for the calibration standards, with regression coefficients (r) above 0.99. The method limit of quantitation (LOQ) was set as the concentration at the signal-to-noise ratio (S/N) of 10. The LOQs of all of the target analytes were 0.003–0.138 ng/mL (Table S4). Concentrations below the LOQs were replaced by the value of LOQs/ $\sqrt{2}$ for statistical analysis.

2.6. Health Risk Assessment. A total of 616 urine samples were included for the calculation of estimated daily intake (EDI, μ g/kg body weight [bw]/day), hazard quotients (HQs), and hazard indexes (HIs). Forty-seven samples were excluded from these calculations due to the lack of body weight. The EDI was calculated according to the following formula:^{31,49}

$$\text{EDI } (\mu\text{g/kg bw/day}) = \frac{C_a (\mu\text{g/L}) \times V (\text{L/day})}{M_b (\text{kg}) \times F}$$

where C_a is the concentration of urinary antibiotics, V is the urine excretion rate, M_b is the body weight, and F is the proportion of the unchanged plus glucuronide-conjugated forms of antibiotics that were excreted through the urine over the total amount of ingested antibiotics. V values were set as 1.2 and 1.6 L/day for women and men aged ≥ 20 years, respectively, and 0.5, 0.7, and 1.2 L/day for subjects aged 2–5, 6–10, and 11–19 years, respectively.^{50,51} M_b can be obtained based on the information in the questionnaires. The F values of all of the antibiotics are provided in Table S5.

The HQ of individual antibiotics and the HI of multiple antibiotics were calculated as follows:^{31,52}

$$\text{HQ} = \text{EDI/ADI}; \text{HI} = \sum \text{HQ}$$

where ADI is the acceptable daily intake for antibiotics based on microbiological effects. The ADI of all of the antibiotics is presented in Table S6. HI or HQ > 1 indicates a potential health risk.

2.7. Statistical Analysis. Data analysis was performed with Origin Pro (Version 2021, OriginLab Inc.) and IBM SPSS Statistics (Version 22.0, SPSS Inc.). A Spearman analysis was used to assess the correlations between the urinary antibiotics. The Mann–Whitney U test or Kruskal–Wallis test was performed to examine the differences in the concentrations of antibiotics among different regions, occupations, genders, and age groups. A multivariable linear regression model was applied to analyze the associations of common log-transformed antibiotic concentrations with the COVID-19 pandemic and the consumption frequency of eggs, milk, and meat. A multinomial logistic regression model was used to determine the association between the overweight and common log-transformed antibiotic concentrations (individual antibiotics with detection frequencies <10% were excluded).⁵³ A p -value of less than 0.05 was considered to be statistically significant.

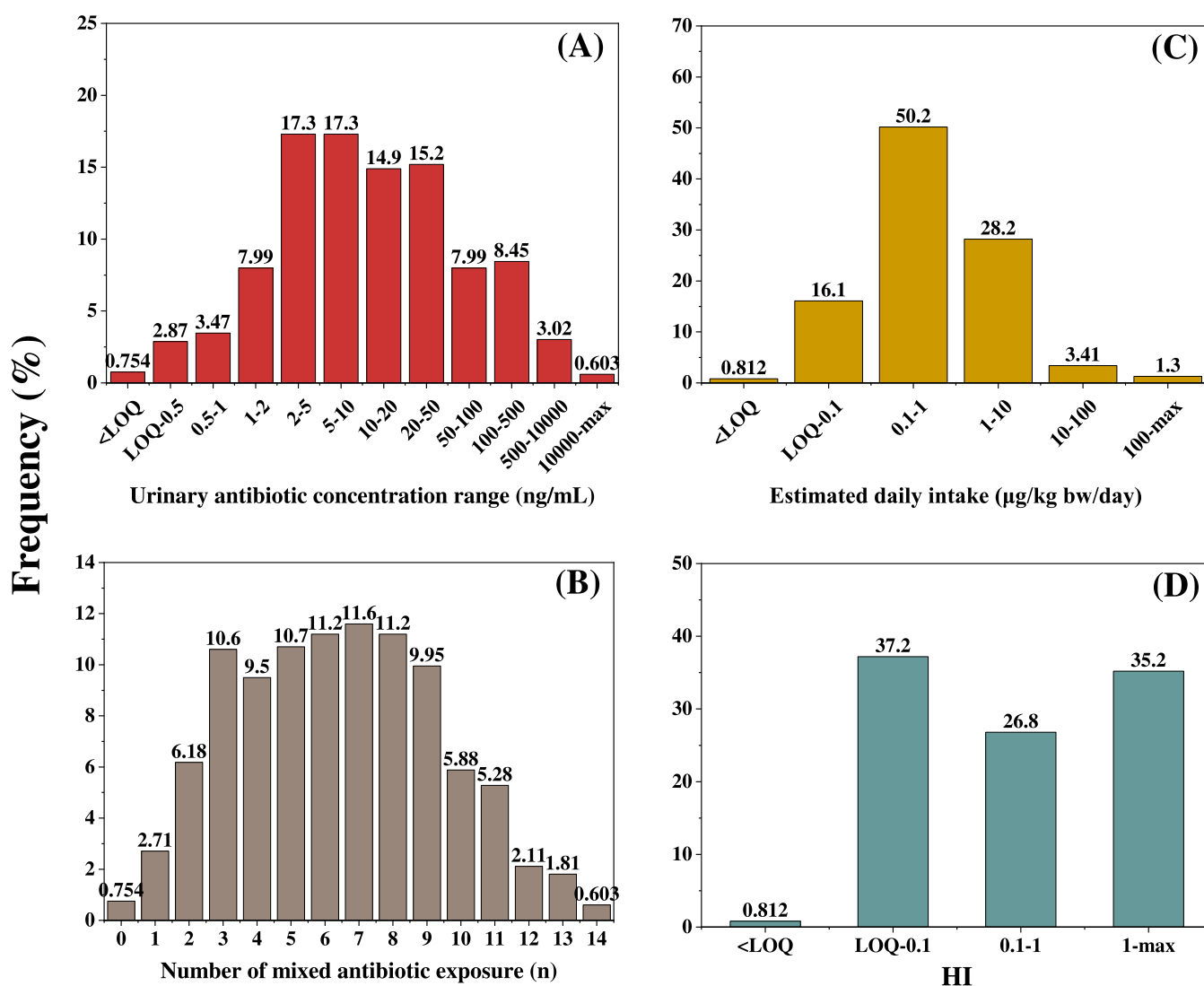


Figure 2. Frequency distribution of the (A) urinary concentrations of 31 antibiotics, (B) number of mixed antibiotic exposure, (C) estimated daily intake of 31 antibiotics, and (D) HIs of above 1.

3. RESULTS AND DISCUSSION

3.1. Impacts of the COVID-19 Pandemic on Urinary Antibiotics. Antibiotics were found in 99.2% of urine samples at a concentration range of not detected (nd) to 357 000 ng/mL, with a median concentration of 10.1 ng/mL (Table 1). The total antibiotic concentrations at nd to 2, 2–50, and 50–357 000 (maximum) ng/mL were detected in 15.1, 64.7, and 20.2% of urine samples, respectively (Figure 2A). This is consistent with the urinary concentration profile of 34 antibiotics in elderly individuals from Anhui Province, China, wherein a detection frequency of 61.8% was observed for 34 antibiotics at concentrations of 2–50 ng/mL.³⁰ Sulfonamides, fluoroquinolones, tetracyclines, amphenicols, β -lactams, macrolides, and lincomycin were found in 86.4, 79.8, 58.2, 39.1, 35.6, 35.6, and 7.24% of urine samples, respectively. Relatively higher detection frequencies (>50.0%) for sulfonamides, fluoroquinolones, and tetracyclines suggest their widespread occurrence in the urine. Fluoroquinolones in the urine were more abundant than sulfonamides and tetracyclines. The median concentration of fluoroquinolones (2.57 ng/mL) in the urine was ~10 times higher than those of sulfonamides (0.273 ng/mL) and tetracyclines (0.244 ng/mL). It has been

reported that fluoroquinolones were the most prescribed antibiotic group in hospitals nationwide in 2017.²⁶ Analogously, the highest urinary level of the antibiotic category in pregnant women in Shanghai, China, was found for fluoroquinolones (1.94 ng/mL).⁴⁶

Three individual antibiotics were detected in more than 50.0% of urine samples, and their median concentrations decreased in order: norfloxacin (0.95 ng/mL) > trimethoprim (0.174 ng/mL) > ofloxacin (0.102 ng/mL) (Figure S1). Trimethoprim,^{30,31,33,34} ofloxacin,^{30,31,33,34} and norfloxacin^{30,46} have been reported as being the most frequently detected antibiotics in urine samples. Several extraordinary concentrations (>1000 ng/mL) were found for roxithromycin (1530 ng/mL), clarithromycin (4880 ng/mL), ofloxacin (20 900 ng/mL), and amoxicillin (357 000 ng/mL), which was likely related to the administration of corresponding antibiotics shortly before sampling (according to the information in the questionnaires).

Spearman's correlation was conducted for individual antibiotics and antibiotic groups in the urine, and the results showed that positive correlations were generally observed among urinary antibiotics, thus indicating their coexistence in

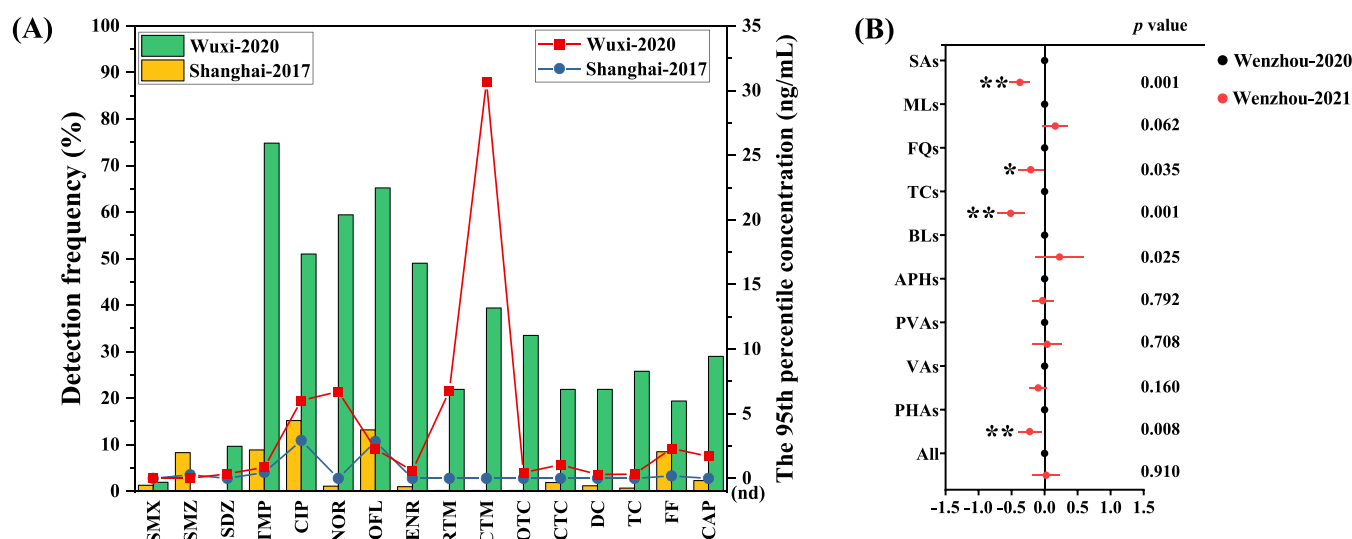


Figure 3. (A) Detection frequencies and the 95th percentile concentrations of urinary antibiotics in general populations from Wuxi in 2020 and Shanghai in 2017. (B) Adjusted regression coefficients (95% CIs) for association of common log-transformed urinary antibiotics with the sampling year. Covariates for adjustment include age, gender, occupation, BMI, and weekly intake of egg, milk, and meat. SMX, sulfamethoxazole; SMZ, sulfamethazine; SDZ, sulfadiazine; TMP, trimethoprim; CIP, ciprofloxacin; NOR, norfloxacin; OFL, ofloxacin; ENR, enrofloxacin; RTM, roxithromycin; CTM, clarithromycin; OTC, oxytetracycline; CTC, chlortetracycline; DC, doxycycline; TC, tetracycline; FF, florfenicol; CAP, chloramphenicol; SAs, sulfonamides; MLs, macrolides; FQs, fluoroquinolones; TCs, tetracyclines; APHs, amphenicols; BLs, β -lactams; PVAs, antibiotics preferred as veterinary antibiotics; VAs, veterinary antibiotics; and PHAs, antibiotics preferred as human antibiotics. * $p < 0.05$; ** $p < 0.01$. The antibiotic group of lincomycin with detection frequency $< 10\%$ was excluded.

local residents (Figure S2). Concurrent exposure to more than one antibiotic was found in 96.5% of the urine samples (Figure 2B). Thirty-two and 37 antibiotics have been detected in aquatic products¹⁶ and surface water¹² in China, respectively. Multiple antibiotics have also been reported in Chinese livestock products, including eggs, milk, and meat.^{14,54,55} Via dietary intake, medication, and other routes, humans can be exposed to multiple antibiotics. The proportion of 96.5% in the general population is obviously higher than those in children (58.3%),³⁴ pregnant women (13.1%),³¹ and adults (15.5%)³³ in Eastern China. This may be attributed to the temporal and regional differences, as well as the lower LOQs, in this study (0.003–0.138 ng/mL) than in previous studies (0.13–6.63 ng/mL).^{31,33,34} In view of the increased online pharmacy sales of medicines,⁵ the common sales of nonprescription antibiotics in pharmacies,^{8,27} and the relatively weak awareness regarding the rational use of antibiotics for customers²⁸ during the COVID-19 pandemic, the action of humans taking more antibiotics to cure diseases may have also contributed to the multiple antibiotic exposures. Higher proportions of people were exposed to 3–9 antibiotics (9.5–11.6%) than 0–2 or 10–14 antibiotics (0.603–6.18%). Furthermore, exposure to 10 or more antibiotics was found in 15.7% of subjects. These results indicate the ubiquitous exposure of general populations to multiple antibiotics in China.

The concentration profiles of five antibiotics (trimethoprim, norfloxacin, ofloxacin, amoxicillin, and ciprofloxacin) with relatively higher detection frequencies and concentrations (Table 1 and Figure S1) were compared with those reported in earlier studies. As shown in Table S7, the detection frequencies of trimethoprim, ofloxacin, and amoxicillin in general populations from four cities in China (the present study) are substantially higher than those in general populations from Korea⁴⁵ and other cities in China.^{30–34,46,47,56} Urinary ofloxacin in this study (the 95th percentile concentration [P95]: 11.7 ng/mL) is ~ 1.26 to 23.4 times higher than those

in other cities in China (0.50–9.25 ng/mL).^{30–34,46,56,57} The P95 concentration of amoxicillin in this study (138 ng/mL) is ~ 5 -fold higher than that found in Anhui Province, Eastern China (30.2 ng/mL).⁵⁶ Our result regarding the relatively higher detection frequencies and P95 concentrations of antibiotics may be attributed to the increased intake of antibiotics by humans during the COVID-19 pandemic.

The association of the COVID-19 pandemic with medication via antibiotics by humans was examined by comparing the urinary antibiotics in the general populations from previous studies and the present study, which collected urine samples in 2017, 2020, and 2021 (Figures 3 and S3). The detection frequencies and the P95 urinary concentrations of some antibiotics (which overlapped in previous studies and the present study) in adults from Wuxi (0–74.8%, nd to 30.7 ng/mL; the present study) were substantially higher than those from Shanghai (which is close to Wuxi) in 2017 (0.1–15.2%, nd to 2.93 ng/mL) (Figure 3A).³³ Moreover, for the antibiotic groups according to usage, the concentrations of PHAs in the urine from Wenzhou in 2021 were significantly lower than those from the same place in 2020 (odds ratio [OR]: -0.248 ; 95% confidence interval [CI]: -0.431 , -0.057 ; $p < 0.01$; Figure 3B). For the antibiotic groups according to the antimicrobial mechanism, similar tendencies were observed for tetracyclines, fluoroquinolones, and sulfonamides ($p < 0.05$). It has been reported that animal-derived food plays a key role in human exposure to antibiotics via dietary intake.^{46,58} Due to the decreasing tendency in antibiotic usage per ton of animal products in China since 2014,²⁵ the rise in concentrations of antibiotics in the urine collected from the general population in the test areas in 2020 (compared to 2017) was likely due to increased intake of antibiotics (self-medication) during the COVID-19 pandemic, especially considering the relatively lower bioavailability of antibiotics through oral uptake than via intravenous injection.⁶ Moreover, consistent with the above-mentioned results, relatively higher

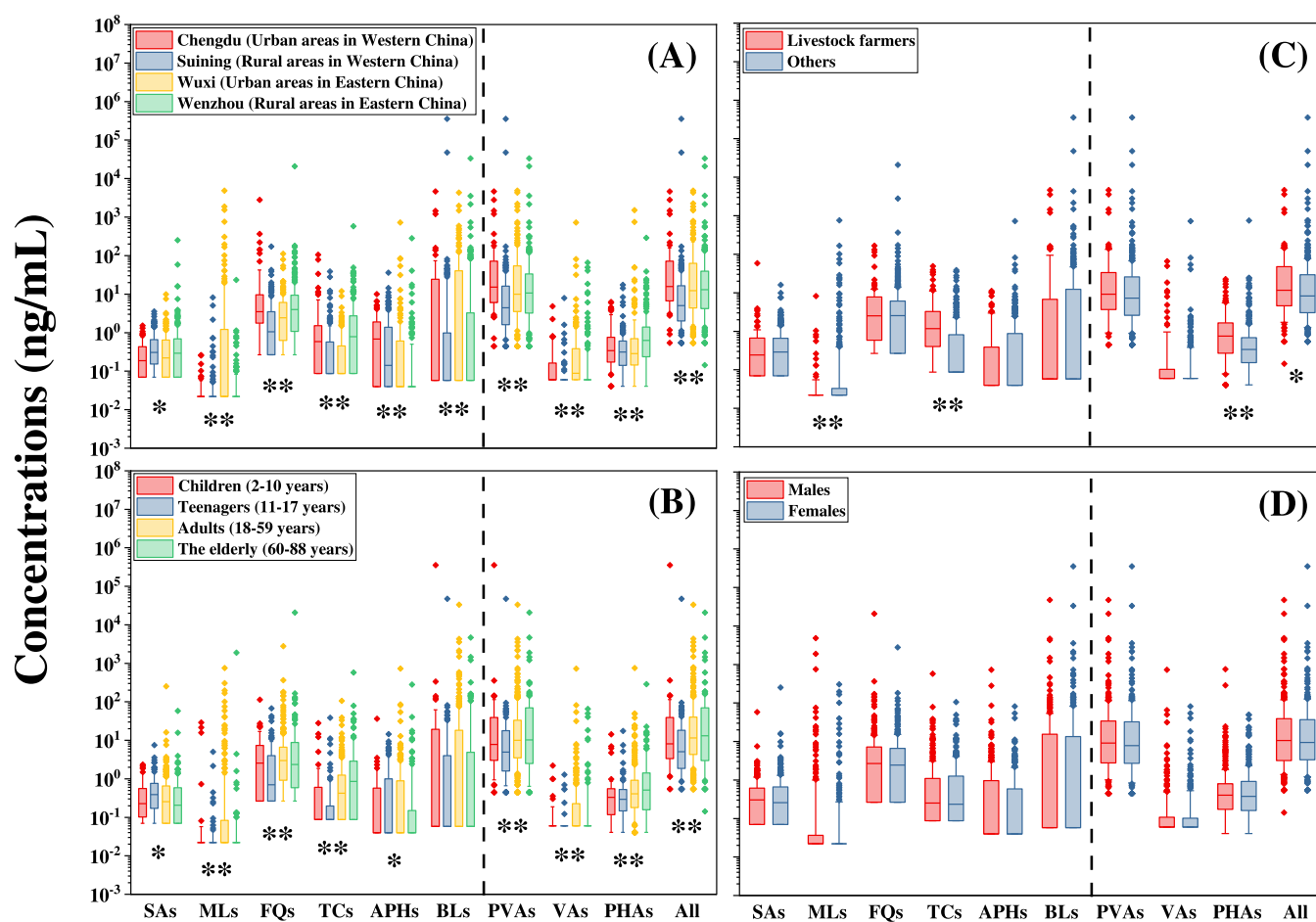


Figure 4. Urinary antibiotic concentrations are categorized by (A) region, (B) age, (C) occupation, and (D) gender. The bottom, middle, and top lines in the box represent the 25th, 50th, and 75th percentile concentrations, respectively. The lower and upper whiskers denote the 10th and 90th percentile concentrations, respectively. The dots refer to outliers. SAs, sulfonamides; MLs, macrolides; FQs, fluoroquinolones; TCs, tetracyclines; APHs, amphenicols; BLs, β -lactams; PVAs, antibiotics preferred as veterinary antibiotics; VAs, veterinary antibiotics; and PHAs, antibiotics preferred as human antibiotics. * $p < 0.05$; ** $p < 0.01$. The antibiotic group of lincomycin with detection frequency $< 10\%$ was excluded.

levels of sulfadiazine, sulfamethoxazole, and sulfamethazine (divided into the sulfonamide group), as well as ofloxacin (divided into the fluoroquinolone group), were found in the receiving rivers of sewage across Shanghai in November 2020 (range: nd to 76.7, 8.83–35.1, 10.9–332, and nd to 40.0 ng/L) than those in November 2014 (nd to 53.9, nd to 26.0, nd to 10.1, and nd to 16.1 ng/L).^{59,60}

3.2. Regional and Demographic-Related Differences in Urinary Antibiotics. Regional differences in the concentrations of antibiotic groups in urine samples from several regions with different confirmed cases of COVID-19 were compared (Figures 4A and S4A and Table S8). The total antibiotic concentrations in the general population from Suining (P95: 59.4 ng/mL) were significantly lower than those from Wuxi, Chengdu, and Wenzhou (747, 709, and 169 ng/mL, respectively; $p < 0.01$). Similar patterns were observed for VAs and PVAs (the antibiotic groups according to usage) ($p < 0.01$). Concentrations of PHAs in Wenzhou (9.92 ng/mL) were substantially higher than those in Chengdu, Wuxi, and Suining (4.11, 7.49, and 1.49 ng/mL, respectively; $p < 0.05$). For the antibiotic groups according to the antimicrobial mechanism, fluoroquinolones and tetracyclines were found at relatively lower levels in urine samples from Suining (14.0 and 2.68 ng/mL, respectively) and Wuxi (15.8 and 1.96 ng/mL, respectively) than in samples from Chengdu (144 and 12.9 ng/

mL, respectively) and Wenzhou (41.2 and 13.7 ng/mL, respectively) ($p < 0.05$). Furthermore, relatively lower levels of β -lactams were found in Suining (44.4 ng/mL) than in Chengdu (144 ng/mL) and Wuxi (315 ng/mL) ($p < 0.05$). These regional differences in urinary concentrations of antibiotic groups are likely associated with the epidemic situations in these areas. Cumulative confirmed domestic cases with COVID-19 in the four involved cities by June 30, 2020, were ordered as follows: Suining (17) $<$ Wuxi (55) $<$ Chengdu (144) $<$ Wenzhou (504 cases) (Figure 1).^{40–42} Elevated urinary concentrations of antibiotics were observed for the residents in the cities with more confirmed COVID-19 cases. This coincides with the above-mentioned fact that the residents in the city with more confirmed cases would purchase antibiotics in online pharmacies rather than going to see doctors in the hospitals for treating diseases during the COVID-19 pandemic, which could induce increased immoderate ingestion of antibiotics.^{5,8,27,28} Consistently, antibiotic use substantially increased along with the confirmed COVID-19 cases in European regions during the pandemic.²⁹

However, the concentrations of PVAs and amphenicols in the urine from Wenzhou (151 and 1.65 ng/mL, respectively) were significantly lower than those found for Chengdu (707 and 5.03 ng/mL, respectively; $p < 0.05$). Analogously, the concentrations of VAs, amphenicols, and β -lactams in the

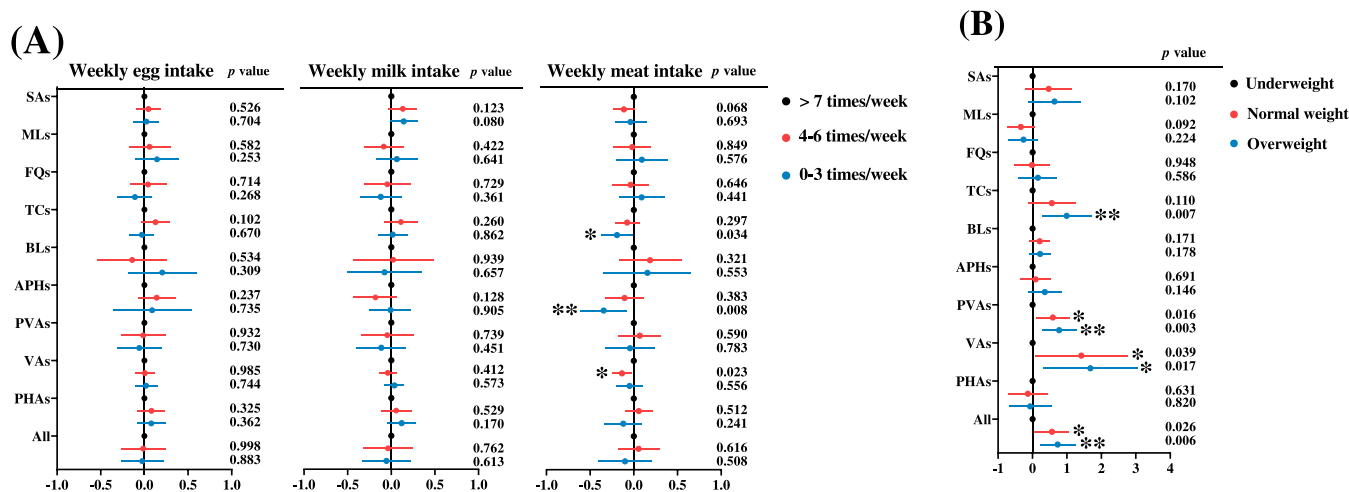


Figure 5. Adjusted regression coefficients (95% CIs) for associations of the common log-transformed urinary antibiotics with the weekly intake of egg, milk, and meat (A) and the overweight (B). Covariates for adjustment include region, age, gender, occupation, BMI, and weekly intake of egg, milk, and meat. SAs, sulfonamides; MLs, macrolides; FQs, fluoroquinolones; TCs, tetracyclines; APHs, amphenicols; BLs, β -lactams; PVAs, antibiotics preferred as veterinary antibiotics; VAs, veterinary antibiotics; and PHAs, antibiotics preferred as human antibiotics. * $p < 0.05$; ** $p < 0.01$. The antibiotic group of lincomycin with detection frequency <10% was excluded.

Wenzhou population (1.35, 1.65, and 130 ng/mL, respectively) were lower than those in the Wuxi population (2.35, 5.16, and 315 ng/mL, respectively; $p < 0.05$). Antibiotic consumption in China is driven by the gross domestic product per capita,^{61,62} therefore, urban residents in Chengdu and Wuxi may be exposed to more antibiotics than rural residents in Wenzhou. Relatively higher levels of antibiotics have been reported in surface water and sediments in urban areas than in rural areas.⁶³ In addition, Eastern–Western differences in urinary antibiotics have been compared. The Wuxi population had relatively higher concentrations of VAs and macrolides (2.35 and 66.3 ng/mL, respectively) than the Chengdu population (0.799 and 0.0864 ng/mL, respectively; $p < 0.05$). This finding can be explained by the relatively higher amounts of antibiotics consumed in Eastern China (38 800 tons in 2013) than in Western China (20 660 tons in 2013).¹¹

The associations of urinary antibiotics with demographic characteristics, including age, gender, and occupation, were investigated (Figures 4 and S4 and Table S8). The elderly individuals had greater urinary total antibiotic concentrations (214 ng/mL) than teenagers (61.8 ng/mL) ($p < 0.01$; Figure 4B and Table S8). Similar tendencies were found for PHAs, VAs, and PVAs, as well as for fluoroquinolones, tetracyclines, and β -lactams ($p < 0.05$). PHAs were also observed at relatively higher levels in the urine of elderly individuals (11.3 ng/mL) than in the urine of children (2.61 ng/mL) ($p < 0.05$). Furthermore, higher urinary levels of tetracyclines were found for the elderly individuals (30.8 ng/mL) than for children (6.51 ng/mL) and adults (5.35 ng/mL) ($p < 0.01$). The relatively weaker immunity of elderly individuals may increase their ingestion of antibiotics to protect against infectious diseases.⁶⁴ Moreover, remarkably higher urinary antibiotics in the elderly individuals in the present study may be explained by the increased self-medication via antibiotics during the COVID-19 pandemic in these areas.

The total antibiotic concentrations in the urine of livestock farmers (169 ng/mL) were significantly higher than those found for other people of different occupations (149 ng/mL) ($p < 0.05$; Figure 4C and Table S8). Similar trends were found for PHAs and tetracyclines ($p < 0.01$). Antibiotics are widely

applied in livestock to prevent and treat infectious diseases and to promote the growth of animals.^{9,10} Via air inhalation and dermal contact, occupational exposure of livestock farm workers to diverse antibiotics is inevitable.⁶⁵ Antibiotics, especially tetracyclines, have been widely detected in manure and dust from pig houses at concentrations of up to the mg/kg level.^{10,66} Elevated levels of tetracyclines in livestock farmers may be attributed to their more frequent working on livestock farms, which was demonstrated by the questionnaires that were collected. No substantial differences in urinary antibiotics were observed between males and females ($p > 0.05$; Figure 4D and Table S8). Analogously, Liu et al.⁶⁷ reported no obvious correlations between gender and serum antibiotic concentrations.

3.3. Sources of Human Exposure to Antibiotics and Health Risk Assessment. In addition to medication, the dietary intake of animal-derived food is also an important source of human exposure to antibiotics.^{32,45,46,58} The dietary factors of human exposure to antibiotics were determined by evaluating the associations of consumption frequencies of animal-derived food (including eggs, milk, and meat) with urinary antibiotics (Figures 5A and S5A). For the antibiotic groups according to usage, lower meat intake was significantly associated with lower concentrations of VAs (the second vs first tertile: OR, -0.13 ; 95% CI, -0.251 , -0.024 ; $p < 0.05$). Similar patterns were observed for amphenicols and tetracyclines (the antibiotic groups according to the antimicrobial mechanism; $p < 0.05$). However, no significant associations were found between the intake of eggs or milk and urinary antibiotics. Higher detection frequencies of antibiotics have been reported in meat than in milk⁵⁴ and eggs.¹⁴ It has been estimated that COVID-19 can survive in the prolonged process of cold-chain transportation.⁶⁸ With concerns of infections by the virus, local meat (instead of imported seafood) was preferred by the residents.⁶⁹ A positive correlation was observed between urinary antibiotics and the intake of meat (rather than eggs or milk), thus suggesting that meat intake may be a pathway of human daily exposure to antibiotics during the COVID-19 pandemic.

The association between BMI and antibiotic exposure was also determined (Figures 5B and S5B). The urinary total antibiotic concentrations were significantly associated with the overweight (the second vs first tertile: OR, 0.563; 95% CI, 0.0665, 1.06; $p < 0.05$; the third vs first tertile: OR, 0.727; 95% CI, 0.206, 1.25; $p < 0.01$). Positive associations were also found between the overweight and urinary VAs and PVAs, as well as tetracyclines ($p < 0.01$). Antibiotics, such as tetracyclines, are widely used as growth promoters in livestock feed for the weight gain of animals.⁷⁰ This may explain the higher exposure to antibiotics in the overweight groups.

The EDIs of total antibiotics for the general population were in the range of nd to 1370 $\mu\text{g/kg bw/day}$, with a median value of 0.451 $\mu\text{g/kg bw/day}$ (Table S9). The EDI range of nd to 0.1 was found in 16.9% of the general population, and a decreasing trend was observed in the percentage of the general population in the EDI ranges of 0.1–1, 1–10, 10–100, and 100–1370 $\mu\text{g/kg bw/day}$ (Figure 2C). Extraordinary EDIs of above 100 $\mu\text{g/kg bw/day}$ were observed in 1.3% of people, with ofloxacin and amoxicillin being the major contributors. HIs of >1 were observed in 35.2% of people (Table S10 and Figure 2D), thus suggesting the potential risk of antibiotic-induced adverse effects. For individual antibiotics, amoxicillin presented HQs of >1 in 22.9% of people, followed by ciprofloxacin (7.31%), clarithromycin (4.06%), tylosin (2.11%), roxithromycin (1.30%), and ofloxacin (1.14%). The percentages of HQs of >1 for other antibiotics were generally lower than 1.00%. Twelve individual antibiotics, including oxytetracycline, doxycycline, tetracycline, chloramphenicol, florfenicol, erythromycin, ofloxacin, roxithromycin, tylosin, clarithromycin, ciprofloxacin, and amoxicillin, presented HQs of >1 in 0.162, 0.162, 0.162, 0.325, 0.325, 1.14, 1.30, 2.11, 4.06, 7.31, and 22.9% of people, respectively. Overall, the prevalence of antibiotics in humans exhibits health risks, particularly considering multiple antibiotic exposures.

4. ENVIRONMENTAL IMPLICATIONS

Our results have demonstrated that the intake of antibiotics in general populations from several regions in China increased during the COVID-19 pandemic, which was evidenced by the following facts. First, significantly higher levels of urinary antibiotics were observed in the peak period of the pandemic than before the pandemic and during the smooth period of the pandemic ($p < 0.05$). Second, concentrations of antibiotics in the urine increased with the increase in confirmed cases in the four investigated cities ($p < 0.05$). Third, the elderly population had an obviously higher body burden of antibiotics than the other age groups ($p < 0.05$), which may be attributed to the increased self-medication of antibiotics by the elderly population during the pandemic due to their relatively weaker immunity. Fourth, intake of meat (rather than eggs and milk) was associated with a higher body burden of antibiotics in these areas, which was different from the previous study that demonstrated that a higher intake of eggs and milk was related to higher urinary antibiotic levels.⁴⁶ During the COVID-19 pandemic, local residents generally ate more meat than seafood (especially imported cold seafood), which was a result of the concern of infection by COVID-19 being transmitted via cold seafood.⁶⁹ It has been reported that improper consumption of antibiotics accounted for 79–96% of the total consumption of antibiotics in the European Region during the COVID-19 pandemic.²⁹ This suggests that the human body burden of

antibiotics in countries other than China may have also increased during the pandemic.

Several uncertainties existed in our estimation of exposure. First, urinary excretion proportions of sulfaquinoxaline, sulfachlorpyridazine, enrofloxacin, tylosin, and florfenicol from pigs were adopted for the exposure calculation due to a lack of human pharmacokinetic data of antibiotics. Second, discussions about the impacts of the COVID-19 pandemic on human exposure to antibiotics were based on urine samples from several cities, and further studies on a large scale of samples are needed. Third, due to the fact that foods such as meat, eggs, and milk contribute to the exposure of local residents to antibiotics, the measurement of the concentrations of antibiotics in various local food would facilitate a better understanding of the effects of dietary intake on the human body burden of such chemicals in these areas during the pandemic. Collectively, our findings highlight how and to what extent the COVID-19 pandemic influences the intake of antibiotics by humans, which provides an important emphasis for the prudent local medication of antibiotics, in terms of co-exposure to multiple antibiotics and resultant health risks in bacterial resistance.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.1c07655>.

Supplementary text, tables, and figures show the classification of urine samples, optimized MS/MS parameters, QA/QC details, urine excretion rates of antibiotics, exposure and risk assessment of antibiotics for general populations, composition profiles, and correlation analysis of urinary antibiotics; comparisons of urinary antibiotics in this study with previous studies, as well as regional and demographics-related differences in antibiotic concentrations and the association of urinary antibiotics with the sampling year, the weekly intake of eggs, milk, and meat, and the overweight (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Chunyang Liao – State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China; Hubei Key Laboratory of Environmental and Health Effects of Persistent Toxic Substances, School of Environment and Health, Jiangnan University, Wuhan, Hubei 430056, China; School of Environment, Hangzhou Institute for Advanced Study, UCAS, Hangzhou, Zhejiang 310024, China; College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, China; orcid.org/0000-0003-2846-6614; Email: cyliao@rcees.ac.cn

Authors

Yu Hu – State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China; College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, China

Xianping Wei – State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China; Hubei Key Laboratory of Environmental and Health Effects of Persistent Toxic Substances, School of Environment and Health, Jiangnan University, Wuhan, Hubei 430056, China

Qingqing Zhu – State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China; College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, China

Lingxiangyu Li – School of Environment, Hangzhou Institute for Advanced Study, UCAS, Hangzhou, Zhejiang 310024, China; orcid.org/0000-0002-2611-380X

Guibin Jiang – State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China; Hubei Key Laboratory of Environmental and Health Effects of Persistent Toxic Substances, School of Environment and Health, Jiangnan University, Wuhan, Hubei 430056, China; School of Environment, Hangzhou Institute for Advanced Study, UCAS, Hangzhou, Zhejiang 310024, China; College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, China; orcid.org/0000-0002-6335-3917

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acs.est.1c07655>

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Key Research and Development Program of China (2019YFC1604802 and 2018YFC1801602), the National Natural Science Foundation of China (21806172 and 22093051), and the K.C. Wong Education Foundation of China (GJTD-2020-03).

REFERENCES

- (1) World Health Organization. Coronavirus Disease (COVID-19) Situation Reports. <https://covid19.who.int/> (accessed November 3, 2021).
- (2) Flaxman, S.; Mishra, S.; Gandy, A.; Unwin, H. J. T.; Mellan, T. A.; Coupland, H.; Whittaker, C.; Zhu, H.; Berah, T.; Eaton, J. W.; Monod, M.; Ghani, A. C.; Donnelly, C. A.; Riley, S.; Vollmer, M. A. C.; Ferguson, N. M.; Okell, L. C.; Bhatt, S.; et al. Estimating the effects of non-pharmaceutical interventions on COVID-19 in Europe. *Nature* **2020**, *584*, 257–261.
- (3) Hao, X.; Cheng, S.; Wu, D.; Wu, T.; Lin, X.; Wang, C. Reconstruction of the full transmission dynamics of COVID-19 in Wuhan. *Nature* **2020**, *584*, 420–424.
- (4) Worobey, M.; Pekar, J.; Larsen, B. B.; Nelson, M. I.; Hill, V.; Joy, J. B.; Rambaut, A.; Suchard, M. A.; Wertheim, J. O.; Lemey, P. The emergence of SARS-CoV-2 in Europe and North America. *Science* **2020**, *370*, 564–570.
- (5) Ji, J. Variabilities and invariabilities in the pharmaceutical industry in the post-epidemic period. *Mod. Commercial Bank* **2021**, *27*, 72–77.
- (6) (a) Hinderliter, P.; Saghir, S. A. Pharmacokinetics. In *Encyclopedia of Toxicology*, 3rd ed.; Wexler, P., Ed.; Academic Press, Elsevier: Netherlands, 2014; pp 849–855. (b) ICRP (International Commission on Radiological Protection). *Basic Anatomical and*

Physiological Data for Use in Radiological Protection: Reference Values; ICRP Publication 89; Pergamon Press: Oxford, 2003; p 161.

(7) Fang, Y. China should curb non-prescription use of antibiotics in the community. *BMJ* **2014**, *348*, 1756–1833.

(8) Zhang, T.; Lambert, H.; Zhao, L.; Liu, R.; Shen, X.; Wang, D.; Cabral, C. Antibiotic stewardship in retail pharmacies and the access-excess challenge in China: A policy review. *Antibiotics* **2022**, *11*, 141.

(9) Sarmah, A. K.; Meyer, M. T.; Boxall, A. B. A. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere* **2006**, *65*, 725–759.

(10) Zhou, L. J.; Ying, G. G.; Liu, S.; Zhang, R. Q.; Lai, H. J.; Chen, Z. F.; Pan, C. G. Excretion masses and environmental occurrence of antibiotics in typical swine and dairy cattle farms in China. *Sci. Total Environ.* **2013**, *444*, 183–195.

(11) Zhang, Q. Q.; Ying, G. G.; Pan, C. G.; Liu, Y. S.; Zhao, J. L. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: Source analysis, multimedia modeling, and linkage to bacterial resistance. *Environ. Sci. Technol.* **2015**, *49*, 6772–6782.

(12) Liu, X.; Lu, S.; Guo, W.; Xi, B.; Wang, W. Antibiotics in the aquatic environments: A review of lakes, China. *Sci. Total Environ.* **2018**, *627*, 1195–1208.

(13) Yang, Y.; Song, W.; Lin, H.; Wang, W.; Du, L.; Xing, W. Antibiotics and antibiotic resistance genes in global lakes: A review and meta-analysis. *Environ. Int.* **2018**, *116*, 60–73.

(14) Yang, Y.; Qiu, W.; Li, Y.; Liu, L. Antibiotic residues in poultry food in Fujian Province of China. *Food Addit. Contam. B* **2020**, *13*, 177–184.

(15) Li, J. Y.; Wen, J.; Chen, Y.; Wang, Q.; Yin, J. Antibiotics in cultured freshwater products in Eastern China: Occurrence, human health risks, sources, and bioaccumulation potential. *Chemosphere* **2021**, *264*, No. 128441.

(16) Liu, X.; Steele, J. C.; Meng, X. Z. Usage, residue, and human health risk of antibiotics in Chinese aquaculture: A review. *Environ. Pollut.* **2017**, *223*, 161–169.

(17) Yilmaz, Ç.; Ozcengiz, G. Antibiotics: Pharmacokinetics, toxicity, resistance and multidrug efflux pumps. *Biochem. Pharmacol.* **2017**, *133*, 43–62.

(18) Luo, Y.; Mao, D.; Rysz, M.; Zhou, Q.; Zhang, H.; Xu, L.; Alvarez, P. J. J. Trends in antibiotic resistance genes occurrence in the Haihe River, China. *Environ. Sci. Technol.* **2010**, *44*, 7220–7225.

(19) Vangay, P.; Ward, T.; Gerber, J. S.; Knights, D. Antibiotics, pediatric dysbiosis, and disease. *Cell Host Microbe* **2015**, *17*, 553–564.

(20) Zhang, L.; Huang, Y.; Zhou, Y.; Buckley, T.; Wang, H. H. Antibiotic administration routes significantly influence the levels of antibiotic resistance in gut microbiota. *Antimicrob. Agents Chemother.* **2013**, *57*, 3659–3666.

(21) Zhou, Y.; Li, Y.; Zhang, L.; Wu, Z.; Huang, Y.; Yan, H.; Zhong, J.; Wang, L. J.; Abdullah, H. M.; Wang, H. H. Antibiotic administration routes and oral exposure to antibiotic resistant bacteria as key drivers for gut microbiota disruption and resistome in poultry. *Front. Microbiol.* **2020**, *11*, 1319.

(22) Bassetti, M.; Poulakou, G.; Ruppe, E.; Bouza, E.; Van Hal, S. J.; Brink, A. Antimicrobial resistance in the next 30 years, humankind, bugs and drugs: a visionary approach. *Intensive Care Med.* **2017**, *43*, 1464–1475.

(23) Ministry of Health of the People's Republic of China. The Administrative Measures for Clinical Use of Antimicrobial Agents. http://www.gov.cn/govweb/gongbao/content/2012/content_2201890.htm (accessed October 20, 2021).

(24) National Health Commission of People's Republic of China. The National Action Plan for Inhibiting Bacterial Resistance (2016–2020). http://www.gov.cn/xinwen/2016-08/25/content_5102348.htm (accessed October 20, 2021).

(25) National Health Commission of the People's Republic of China. Report on the Status of Antimicrobial Management and Bacterial Resistance in China (2018). <http://www.nhc.gov.cn/zygj/s3594/201904/1b5a42f0e326487295b260c813da9b0e.shtml> (accessed October 20, 2021).

- (26) China Animal Health. Report on the Use of Veterinary Antimicrobials in China in 2018, 2019; Vol. 21 12, pp 8–9.
- (27) Chen, J.; Wang, Y.; Chen, X.; Hesketh, T. Widespread illegal sales of antibiotics in Chinese pharmacies - a nationwide cross-sectional study. *Antimicrob. Resist. Infect. Control* **2020**, *9*, 12.
- (28) Li, P.; Hayat, K.; Shi, L.; Lambojon, K.; Saeed, A.; Aziz, M. M.; Liu, T.; Ji, S.; Gong, Y.; Feng, Z.; Jiang, M.; Ji, W.; Yang, C.; Chang, J.; Fang, Y. Knowledge, attitude, and practices of antibiotics and antibiotic resistance among Chinese pharmacy customers: A multi-center survey study. *Antibiotics* **2020**, *9*, 184.
- (29) World Health Organization (WHO). Preventing the COVID-19 Pandemic from Causing An Antibiotic Resistance Catastrophe. <https://www.euro.who.int/en/health-topics/disease-prevention/antimicrobial-resistance/news/news/2020/11/preventing-the-covid-19-pandemic-from-causing-an-antibiotic-resistance-catastrophe> (accessed October 20, 2021).
- (30) Zhu, Y.; Liu, K.; Zhang, J.; Liu, X.; Yang, L.; Wei, R.; Wang, S.; Zhang, D.; Xie, S.; Tao, F. Antibiotic body burden of elderly Chinese population and health risk assessment: A human biomonitoring-based study. *Environ. Pollut.* **2020**, *256*, No. 113311.
- (31) Wang, H.; Wang, N.; Qian, J.; Hu, L.; Huang, P.; Su, M.; Xin, Y.; Fu, C.; Jiang, F.; Qi, Z.; Ying, Z.; Lin, H.; He, G.; Yue, C.; Jiang, Q. Urinary Antibiotics of pregnant women in eastern China and cumulative health risk assessment. *Environ. Sci. Technol.* **2017**, *51*, 3518–3525.
- (32) Wang, H.; Tang, C.; Yang, J.; Wang, N.; Jiang, F.; Xia, Q.; He, G.; Chen, Y.; Jiang, Q. Predictors of urinary antibiotics in children of Shanghai and health risk assessment. *Environ. Int.* **2018**, *121*, 507–514.
- (33) Wang, H.; Yang, J.; Yu, X.; Zhao, G.; Zhao, Q.; Wang, N.; Jiang, Y.; Jiang, F.; He, G.; Chen, Y.; Zhou, Z.; Jiang, Q. Exposure of adults to antibiotics in a Shanghai suburban area and health risk assessment: A biomonitoring-based study. *Environ. Sci. Technol.* **2018**, *52*, 13942–13950.
- (34) Wang, H.; Wang, B.; Zhao, Q.; Zhao, Y.; Fu, C.; Feng, X.; Wang, N.; Su, M.; Tang, C.; Jiang, F.; Zhou, Y.; Chen, Y.; Jiang, Q. Antibiotic body burden of Chinese school children: A multisite biomonitoring-based study. *Environ. Sci. Technol.* **2015**, *49*, 5070–5079.
- (35) Zhou, L. J.; Ying, G. G.; Liu, S.; Zhao, J. L.; Yang, B.; Chen, Z. F.; Lai, H. J. Occurrence and fate of eleven classes of antibiotics in two typical wastewater treatment plants in South China. *Sci. Total Environ.* **2013**, *452–453*, 365–376.
- (36) National Health Commission of the People's Republic of China. Coronavirus Disease (COVID-19) Situation Reports. http://www.nhc.gov.cn/xcs/yqtb/list_gzbd_23.shtml (accessed January 18, 2022).
- (37) Ministry of Education of the People's Republic of China. Ensure a Safe, Normal and Comprehensive Start of Schools. http://www.moe.gov.cn/fbh/live/2020/52320/mtbd/202008/t20200828_481679.html (accessed January 18, 2022).
- (38) CCTV. China's Efforts Against the COVID-19 was Appreciated by WHO. <https://tv.cctv.com/2020/09/08/VIDE59bjrpB4IVxChUVaf3Qn200908.shtml> (accessed January 18, 2022).
- (39) National Health Commission of the People's Republic of China. Vaccination Status of COVID-19. <http://www.nhc.gov.cn/xcs/yqjzqk/202105/d0846315c0eb47398c688460e74a88ee.shtml> (accessed January 18, 2022).
- (40) Health Commission of Wenzhou. Coronavirus Disease (COVID-19) Situation Reports. <http://wjw.wenzhou.gov.cn/col/col1209919/index.html> (accessed October 20, 2021).
- (41) Health Commission of Sichuan Province. Coronavirus Disease (COVID-19) Situation Reports. <http://wsjkw.sc.gov.cn/scwsjkw/gzbd01/ztwzlmgl.shtml> (accessed October 20, 2021).
- (42) Health Commission of Wuxi. Coronavirus Disease (COVID-19) Situation Reports. <http://wjw.wuxi.gov.cn/gggs/zxfxdj/index.shtml> (accessed October 20, 2021).
- (43) Worsham, L.; Markewitz, D.; Nibbelink, N. P.; West, L. T. A comparison of three field sampling methods to estimate soil carbon content. *Forest Sci.* **2012**, *58*, 513–522.
- (44) Azad, M. B.; Bridgman, S. L.; Becker, A. B.; Kozyrskyj, A. L. Infant antibiotic exposure and the development of childhood overweight and central adiposity. *Int. J. Obesity* **2014**, *38*, 1290–1298.
- (45) Ji, K.; Kho, Y.; Park, C.; Paek, D.; Ryu, P.; Paek, D.; Kim, M.; Kim, P.; Choi, K. Influence of water and food consumption on inadvertent antibiotics intake among general population. *Environ. Res.* **2010**, *110*, 641–649.
- (46) Zeng, X.; Zhang, L.; Chen, Q.; Yu, K.; Zhao, S.; Zhang, L.; Zhang, J.; Zhang, W.; Huang, L. Maternal antibiotic concentrations in pregnant women in Shanghai and their determinants: A biomonitoring-based prospective study. *Environ. Int.* **2020**, *138*, No. 105638.
- (47) Li, N.; Ho, K. W. K.; Ying, G. G.; Deng, W. J. Veterinary antibiotics in food, drinking water, and the urine of preschool children in Hong Kong. *Environ. Int.* **2017**, *108*, 246–252.
- (48) Wang, H. X.; Wang, B.; Zhou, Y.; Jiang, Q. W. Rapid and sensitive screening and selective quantification of antibiotics in human urine by two-dimensional ultraperformance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. *Anal. Bioanal. Chem.* **2014**, *406*, 8049–8058.
- (49) Wang, B.; Wang, H.; Zhou, W.; Chen, Y.; Zhou, Y.; Jiang, Q. Urinary excretion of phthalate metabolites in school Children of China: Implication for cumulative risk assessment of phthalate exposure. *Environ. Sci. Technol.* **2015**, *49*, 1120–1129.
- (50) Liao, C.; Liu, F.; Alomirah, H.; Vu Duc, L.; Mohd, M. A.; Moon, H. B.; Nakata, H.; Kannan, K. Bisphenol S in Urine from the United States and Seven Asian Countries: Occurrence and Human Exposures. *Environ. Sci. Technol.* **2012**, *46*, 6860–6866.
- (51) ICRP (International Commission on Radiological Protection). *Basic Anatomical and Physiological Data for Use in Radiological Protection: Reference Values*; ICRP Publication 89; Pergamon Press: Oxford, 2003; p 161.
- (52) Koch, H. M.; Wittassek, M.; Bruening, T.; Angerer, J.; Heudorf, U. Exposure to phthalates in 5-6 years old primary school starters in Germany-A human biomonitoring study and a cumulative risk assessment. *Int. J. Hyg. Environ. Health* **2011**, *214*, 188–195.
- (53) Wang, H.; Wang, N.; Wang, B.; Fang, H.; Fu, C.; Tang, C.; Jiang, F.; Zhou, Y.; He, G.; Zhao, Q.; Chen, Y.; Jiang, Q. Antibiotics detected in urines and adipogenesis in school children. *Environ. Int.* **2016**, *89–90*, 204–211.
- (54) Wang, H.; Ren, L.; Yu, X.; Hu, J.; Chen, Y.; He, G.; Jiang, Q. Antibiotic residues in meat, milk and aquatic products in Shanghai and human exposure assessment. *Food Control* **2017**, *80*, 217–225.
- (55) Zheng, N.; Wang, J.; Han, R.; Xu, X.; Zhen, Y.; Qu, X.; Sun, P.; Li, S.; Yu, Z. Occurrence of several main antibiotic residues in raw milk in 10 provinces of China. *Food Addit. Contam. B* **2013**, *6*, 84–89.
- (56) Zhang, J.; Liu, X.; Zhu, Y.; Yang, L.; Sun, L.; Wei, R.; Chen, G.; Wang, Q.; Sheng, J.; Liu, A.; Tao, F.; Liu, K. Antibiotic exposure across three generations from Chinese families and cumulative health risk. *Ecotoxicol. Environ. Saf.* **2020**, *191*, No. 110237.
- (57) Geng, M.; Liu, K.; Huang, K.; Zhu, Y.; Ding, P.; Zhang, J.; Wang, B.; Liu, W.; Han, Y.; Gao, H.; Wang, S.; Chen, G.; Wu, X.; Tao, F. Urinary antibiotic exposure across pregnancy from Chinese pregnant women and health risk assessment: Repeated measures analysis. *Environ. Int.* **2020**, *145*, No. 106164.
- (58) Wang, H.; Wang, N.; Wang, B.; Zhao, Q.; Fang, H.; Fu, C.; Tang, C.; Jiang, F.; Zhou, Y.; Chen, Y.; Jiang, Q. Antibiotics in drinking water in Shanghai and their contribution to antibiotic exposure of school children. *Environ. Sci. Technol.* **2016**, *50*, 2692–2699.
- (59) Li, D.; Shao, H.; Huo, Z.; Xie, N.; Gu, J.; Xu, G. Typical antibiotics in the receiving rivers of direct-discharge sources of sewage across Shanghai: occurrence and source analysis. *RSC Adv.* **2021**, *11*, 21579–21587.
- (60) Wu, M. H.; Que, C. J.; Xu, G.; Sun, Y. F.; Ma, J.; Xu, H.; Sun, R.; Tang, L. Occurrence, fate and interrelation of selected antibiotics

in sewage treatment plants and their receiving surface water. *Ecotoxcol. Environ. Saf.* **2016**, *132*, 132–139.

(61) Bu, Q.; Wang, B.; Huang, J.; Bhattacharya Liu, K.; Deng, S.; Wang, Y.; Yu, G. Estimating the use of antibiotics for humans across China. *Chemosphere* **2016**, *144*, 1384–1390.

(62) Klein, E. Y.; Van Boeckel, T. P.; Martinez, E. M.; Pant, S.; Gandra, S.; Levin, S. A.; Goossens, H.; Laxminarayan, R. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proc. Natl. Acad. Sci. U.S.A.* **2018**, *115*, E3463–E3470.

(63) Yang, J.; Huang, Y.; Chen, Y.; Hassan, M.; Zhang, X.; Zhang, B.; Gin, K. Y. H.; He, Y. Multi-phase distribution, spatiotemporal variation and risk assessment of antibiotics in a typical urban-rural watershed. *Ecotoxicol. Environ. Saf.* **2020**, *206*, No. 111156.

(64) Yoshikawa, T. T. Epidemiology and unique aspects of aging and infectious diseases. *Clin. Infect. Dis.* **2000**, *30*, 931–933.

(65) Paul, R.; Gerling, S.; Berger, M.; Bluemlein, K.; Jaeckel, U.; Schuchardt, S. Occupational exposure to antibiotics in poultry feeding farms. *Ann. Work Exposure Health* **2019**, *63*, 821–827.

(66) Hamscher, G.; Pawelzick, H. T.; Sczesny, S.; Nau, H.; Hartung, J. Antibiotics in dust originating from a pig-fattening farm: A new source of health hazard for farmers? *Environ. Health Perspect.* **2003**, *111*, 1590–1594.

(67) Liu, S.; Zhao, G.; Zhao, H.; Zhai, G.; Chen, J.; Zhao, H. Antibiotics in a general population: Relations with gender, body mass index (BMI) and age and their human health risks. *Sci. Total Environ.* **2017**, *599–600*, 298–304.

(68) Hu, L.; Gao, J.; Yao, L.; Zeng, L.; Liu, Q.; Zhou, Q.; Zhang, H.; Lu, D.; Fu, J.; Liu, Q. S.; Li, M.; Zhao, X.; Hou, X.; Shi, J.; Liu, L.; Guo, Y.; Wang, Y.; Ying, G. G.; Cai, Y.; Yao, M.; Cai, Z.; Wu, Y.; Qu, G.; Jiang, G. Evidence of foodborne transmission of the Coronavirus (COVID-19) through the animal products food supply chain. *Environ. Sci. Technol.* **2021**, *55*, 2713–2716.

(69) Chinese Market Regulation News. Tips on Being Careful with Cold-Chain Food were Announced by Many Places. http://www.cmrn.com.cn/news/content/2021-01/07/content_134423.html (accessed October 20, 2021).

(70) Iizumi, T.; Battaglia, T.; Ruiz, V.; Perez, G. I. P. Gut microbiome and antibiotics. *Arch. Med. Res.* **2017**, *48*, 727–734.